

**Supplemental Materials for:**

A simple strategy for the creation of a recombinant lectin microarray

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## Supplemental Tables

**Table S1:** Primers Used for Cloning.

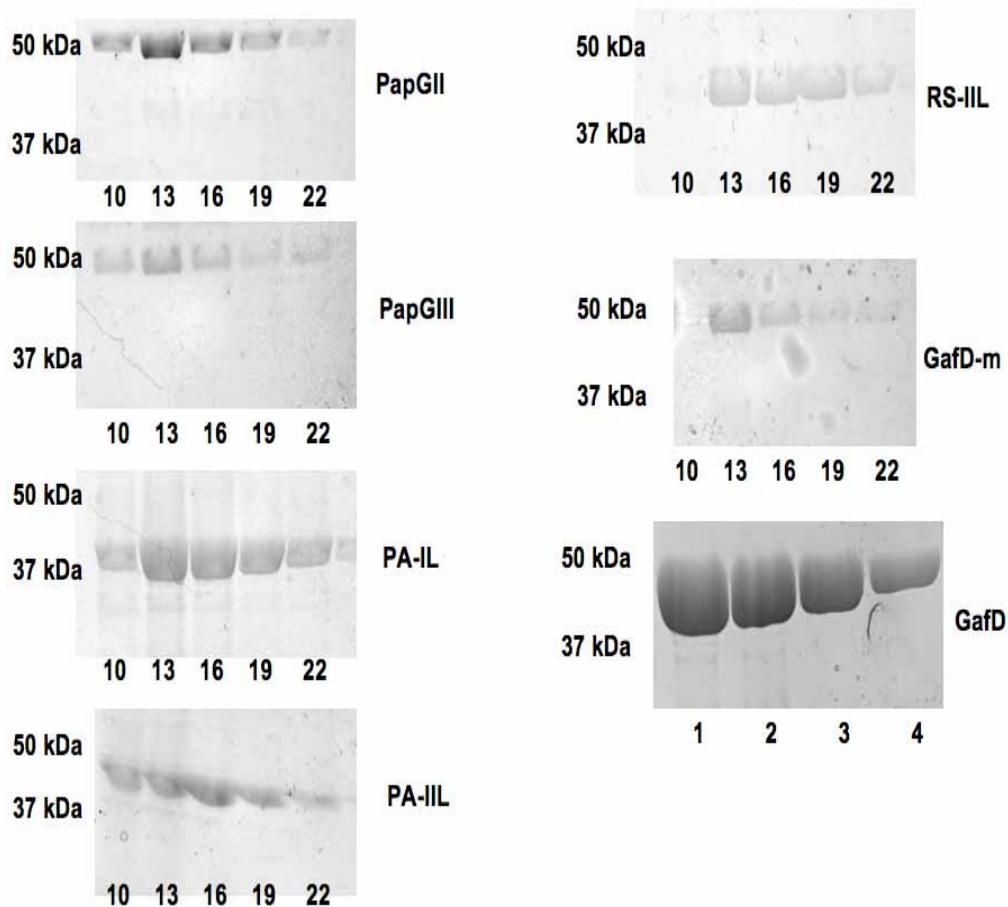
Lectin	Forward primer	Reverse primer
GafD	5' GAC GAC GAC AAG ATG GTT TCA TTT ATT GGC AGT ACG GAG 3'	5' GAG GAG AAG CCC GGT TTA TGT TAC TGT GTC ATT CAG CGT AAA TGG 3'
PA-IL	5' GAC GAC GAC AAG ATG GCT TGG AAA GGT GAG GTT C 3'	5' GAG GAG AAG CCC GGT TCA GGA CTG ATC CTT TCC AAT ATT GAC 3'
PA-IIL	5' GAC GAC GAC AAG ATG GCA ACA CAA GGA GTG TTC ACC 3'	5' GAG GAG AAG CCC GGT CTA GCC GAG CGG CCA GTT GAT 3'
PapGII	5' GAC GAC GAC AAG ATG TGG AAT AAT ATT GTC TTT TAC TCC CTT GG 3'	5' GAG GAG AAG CCC GGT TTA TCC GCC GAT ATT CTT AAA TAA GAA TAA CAT TTC 3'
PapGIII	5' GAC GAC GAC AAG ATG GCA AAC CAG AGT ACA ATG TTT TAC 3'	5' GAG GAG AAG CCC GGT TCA TCC TCC GGT ATT TTT AAT TGA G 3'
RS-IIL	5' GAC GAC GAC AAG ATG GCT CAG CAA GGT GTA TTC 3'	5' GAG GAG AAG CCC GGT TCA GCC CAG CGG CCA GTT 3'
GafD-m	5' GAC GAC GAC AAG ATG GTT TCA TTT ATT GGC AGT ACG GAG 3'	5' GAG GAG AAG CCC GGT TTA TGT TAC TGT GTC ATT CAG CGT AAA TGG 3'

**Table S2:** List of Components on the Array and Abbreviations

<b>Group</b>	<b>Abbreviation</b>	<b>Description</b>
Defined Sugars	3'SLacNAc	Sialyl $\alpha$ 2-3Gal $\beta$ 1-4GlcNAc – BSA (3'SLacNAc)
	6'SLac	Sialyl $\alpha$ 2-3Gal $\beta$ 1-4Glc-APD-HSA (6'SLac)
	Adi	GalNAc $\alpha$ 1-3Gal $\beta$ -BSA (Adi)
	alphaGal	Gal $\alpha$ 1-3Gal $\beta$ 1-4GlcNAc-BSA (aGal)
	Ara5	Ara $\alpha$ 1-5Ara $\alpha$ 1-5Ara $\alpha$ 1-5Ara $\alpha$ 1-5Ara $\alpha$ 1-BSA (Ara5)
	Bdi	Gal $\alpha$ 1-3Gal– BSA (Bdi)
	BG-A	GalNAc $\alpha$ 1-3(Fuca1-2)Gal $\beta$ - BSA [BG-A]
	BG-A1	GalNAc $\alpha$ 1-3(Fuca1-2)Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4(Glc)-APD-HSA (BG-A1)
	BG-B	Gal $\alpha$ 1-3(Fuca1-2)Gal $\beta$ -BSA [BG-B] from EMD
	BG-B (Dextra)	Gal $\alpha$ 1-3(Fuca1-2)Gal $\beta$ -BSA [BG-B] from Dextra
	BG-H1	Fuca1-2Gal $\beta$ 1-3GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ –APD-HSA [BG-H1]
	BG-H2	Fuca1-2Gal $\beta$ 1-4GlcNAc $\beta$ -HSA (BG-H2)
	Cellobiose	Glc $\beta$ 1-4Glc $\beta$ 1-4Glc $\beta$ -BSA (Cello3)
	Cellotriose	Glc $\beta$ 1-4Glc $\beta$ -BSA (Cellotriose)
	Chito 3	GlcNAc $\beta$ 1-4GlcNAc $\beta$ 1-4GlcNAc $\beta$ -BSA (Chito 3)
	DSLNT	Sia $\alpha$ 2-3Gal $\beta$ 1-3(Sia $\alpha$ 2-6)GlcNAc $\beta$ 1-3Gal $\beta$ 1-BSA (DSLNT)
	Fuc-a	Fuc- $\alpha$ - BSA
	Fuc-b	Fuc- $\beta$ - BSA
	G2M4	Man $\beta$ 1-4(Gal $\alpha$ 1-6)Man $\beta$ 1-4(Gal $\alpha$ 1-6)Man $\beta$ 1-4Man $\beta$ 1-BSA (G2M4)
	GA1	Gal $\beta$ 1-3GalNAc $\beta$ 1-4Gal $\beta$ 1-BSA (GA1tri or asialo-GM1)
	GA2di	GalNAc $\beta$ 1-4Gal $\beta$ - BSA (GA2di)
	Gal3	Gal $\alpha$ 1-3Gal $\beta$ 1-4Gal $\alpha$ -BSA (Gal3)
	Gal-a	Gal- $\alpha$ - BSA
	Gala1-4Galb	Gal $\alpha$ 1-4Gal $\beta$ -CETE-BSA
	Gal-b	Gal- $\beta$ - BSA
	Galb1-6Man-a	Gal $\beta$ 1-6Man- $\alpha$ - BSA
	GalNAc-a	GalNAc- $\alpha$ - BSA
	GalNAca1-6Galb	GalNAc $\alpha$ 1-6Gal $\beta$ -BSA
	GalNAc-b	GalNAc- $\beta$ - BSA
	Gb4	GalNAc $\beta$ 1-3Gal $\alpha$ 1-4Gal $\beta$ 1-BSA (Gb4)
	Glc-a	Glc- $\alpha$ - BSA
	Glca1-6Glca1-4Glca1-4Glcb	Glc $\alpha$ 1-6Glc $\alpha$ 1-4Glc $\alpha$ 1-4Glc $\beta$ -CETE-BSA
	Glc-b	Glc- $\beta$ - BSA
	GlcNAca1-4Galb	GlcNAc $\alpha$ 1-4Gal $\beta$ -BSA
	GlcNAc-b	GlcNAc- $\beta$ - BSA
	GM1	Gal $\beta$ 1-3GalNAc $\beta$ 1-4(Sia $\alpha$ 2-3)Gal $\beta$ -4Glc-HSA (GM1)
GM3	Sialyl $\alpha$ 2-3Gal $\beta$ 1-4Glc-APD-HSA (GM3 )	
Isomaltose	Glc $\alpha$ 1-6Glc $\beta$ -BSA (Isomalt)	
LacNAc	Gal $\beta$ 1-4Glc $\beta$ – BSA (Lac)	
Lactose	Gal $\beta$ 1-4GlcNAc – BSA (LacNAc,)	
LeA	Gal $\beta$ 1-3[Fuca1-4]GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ - BSA (Lea )	
LeB	Fuca1-2Gal $\beta$ 1-3[Fuca1-4]GlcNAc $\beta$ 1-3Gal $\beta$ 1-4Glc $\beta$ -BSA (Leb )	
LeC	Gal $\beta$ 1-3GlcNAc $\beta$ – BSA (LeC)	
LeX (dimeric)	Di-LeX-APE-BSA	
LeX (monomeric)	Gal $\beta$ 1-4[Fuca1-3]GlcNAc-APD-HSA (Lex)	
LeY	Fuca1-2Gal $\beta$ 1-4[Fuca1-3]GlcNAc –HSA (Ley)	

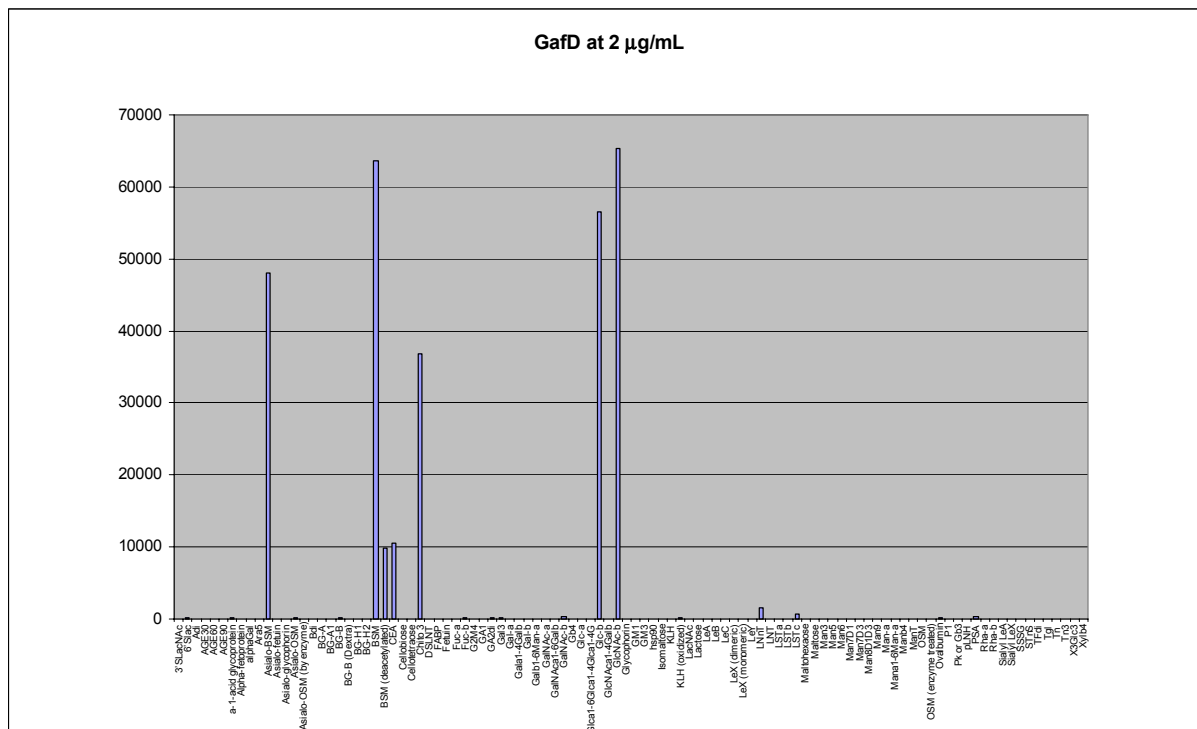
LNnT	Galβ1-4GlcNAcβ1-3Galβ1-BSA (LNnT)
LNT	Galβ1-3GlcNAcβ1-3Galβ-BSA (LNT)
LSTa	Siaα2-3Galβ1-3GlcNAcβ1-3Galβ1-BSA (LSTa)
LSTb	Galβ1-3(Siaα2-6)GlcNAcβ1-3Galβ1-BSA (LSTb)
LSTc	Siaα2-6Galβ1-3GlcNAcβ1-3Galβ1-BSA (LSTc)
Maltohexaose	Glcα1-4Glcα1-4Glcα1-4Glcα1-4Glcα-BSA (Malto5)
Maltose	Glcα1-4Glcβ-BSA (Maltose)
Man3	Manα1-6(Manα1-3)Manβ1-4GlcNAc -BSA (Man3)
Man5	Manα1-6(Manα1-3)Manα1-6(Manα1-3)Manβ1-4GlcNAc-BSA (Man5)
Man6	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAc-BSA (Man6)
Man7D1	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-3)Manβ1-4GlcNAc-BSA (Man7D1)
Man7D3	Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAc-BSA (Man7D3)
Man8D1D3	Manα1-2Manα1-6(Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAc-BSA (Man8D1D3)
Man9	Manα1-2Manα1-6(Manα1-2Manα1-3)Manα1-6(Manα1-2Manα1-2Manα1-3)Manβ1-4GlcNAc-BSA Man9
Man-a	Man-α - BSA
Manα1-6Man-a	Manα1-6Man-α - BSA
Manb4	Manβ1-4Manβ1-4Manβ1-4Manβ1-BSA (Manβ4)
ManT	Manα1-6[Manα1-3]Manβ -BSA [ManT]
P1	Galα1-4Galβ1-4GlcNAc-BSA (P1,)
Pk or Gb3	Galα1-4Galβ1-4Glc-HSA [Pk or Gb3]
pLNH	Galβ1-3GlcNAcβ1-3Galβ1-4GlcNAcβ1-3Galβ1-BSA (pLNH)
Rha-a	Rha-α - BSA
Rha-b	Rha-β - BSA
Sialyl LeA	Siaα-LeA-APD-HSA (SLeA)
Sialyl LeX	Sialylα2-3Galβ1-4[Fucα1-3]GlcNAc - BSA
SSSG	SSSG-BSA
STnS	SerTnSerGly-BSA (STnS)
TFdi	Galβ1-3GalNAcβ - HSA (GA1di)
Tn	GalNAcα1-Thr-Gly-Hex
Tn3	AcTn-Tn-Tn-Gly-Hex-BSA (Tn3)
X3Glc3	Xylα1-6Glcβ1-4(Xylα1-6)Glcβ1-4(Xylα1-6)Glcβ1-BSA (X3Glc3)
Xylb4	Xylβ1-4Xylβ1-4Xylβ1-4Xylβ1-BSA (Xylβ4)
Glycoproteins	AGE30 Advanced glycation endproducts produced from Glc + BSA after 30 days (AGE30)
	AGE60 Advanced glycation endproducts produced from Glc + BSA after 60 days (AGE60)
	AGE90 Advanced glycation endproducts produced from Glc + BSA after 90 days (AGE90)
	Alpha-1-acid glycoprotein alpha1 Acid Glycoprotein
	Alpha-fetoprotein alpha fetoprotein
	Asialo-BSM Asialo-Bovine submaxillary mucin (aBSM, Tn, TF, GlcNAcβ1-3GalNAc)
	Asialo-fetuin asialofetuin
	Asialo-glycophorin asialo-glycophorin (aGn)
	Asialo-OSM asialo-Ovine submaxillary mucin (aOSM)
	Asialo-OSM (enzyme treated) enzyme treated asialo-Ovine submaxillary mucin (almost all Tn)
	BSM Bovine submaxillary mucin (STn, STF, S-GlcNAcβ1-3, ~20% of sialic acid is acetylated at 7,8, or 9)
	BSM (deacetylated) Deacetylated-Bovine submaxillary mucin (deAcBSM)
	CEA carcinoembryonic antigen (CEA)
	FABP Fatty Acid Binding Protein (FABP)
	Fetuin fetuin
	Glycophorin Glycophorin (Gn)
	hsp90 Heat Shock Protein 90 (hsp90)
	KLH Keyhole limpet hemocyanin

	KLH (oxidized)	periodate oxidized Keyhole limpet hemocyanin
	OSM	Ovine submaxillary mucin (94% STn, 4% TF, 2% Fuca1-2Gal $\beta$ 1-3GalNAc)
	OSM (enzyme treated)	enzyme treated Ovine submaxillary mucin (almost all STn)
	Ovalbumin	ovalbumin
	PSA	Prostate Specific Antigen (PSA)
	Tgl	Thyroglobulin (Tgl)
Controls	BSA	Bovine serum albumin
	HSA	Human serum albumin (isolated from serum)
	rHSA	Human serum albumin (recombinant)
	Cy3	Cy3-labeled BSA
	Cy5	Cy5-labeled BSA

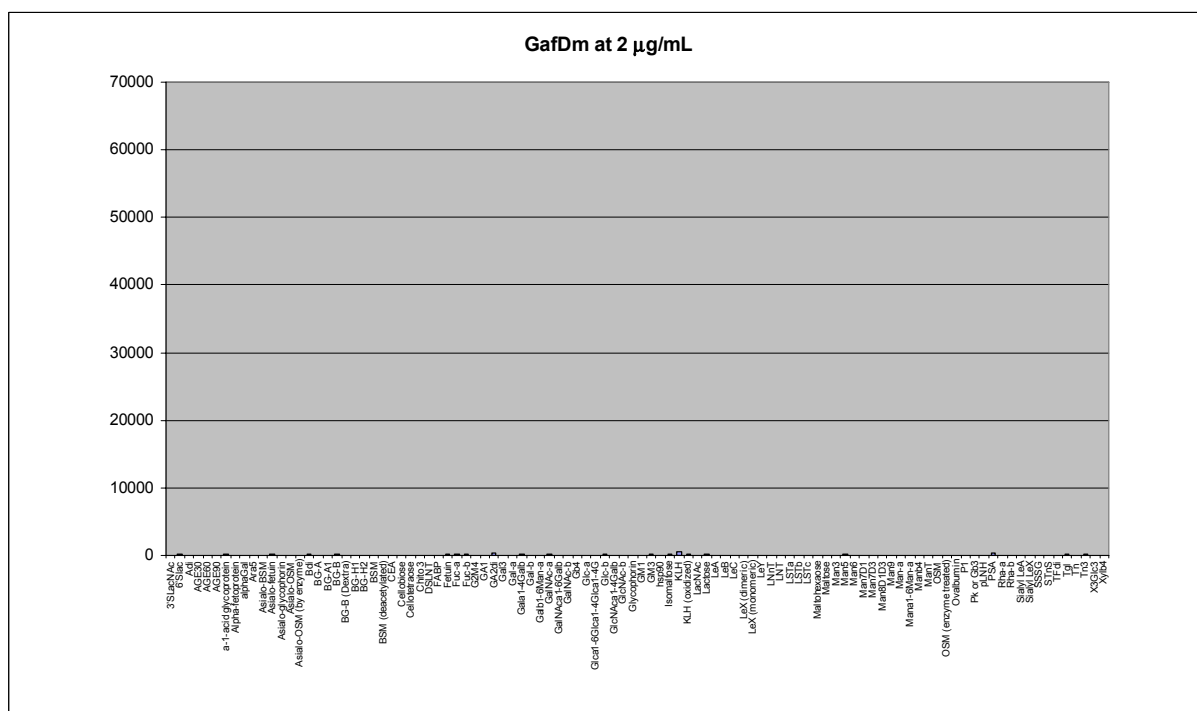


**Figure S1** Expression of the recombinant lectin panel. 10% SDS-PAGE stained with Coomassie Blue showing the elution fractions collected during purification as described in the Experimental section.

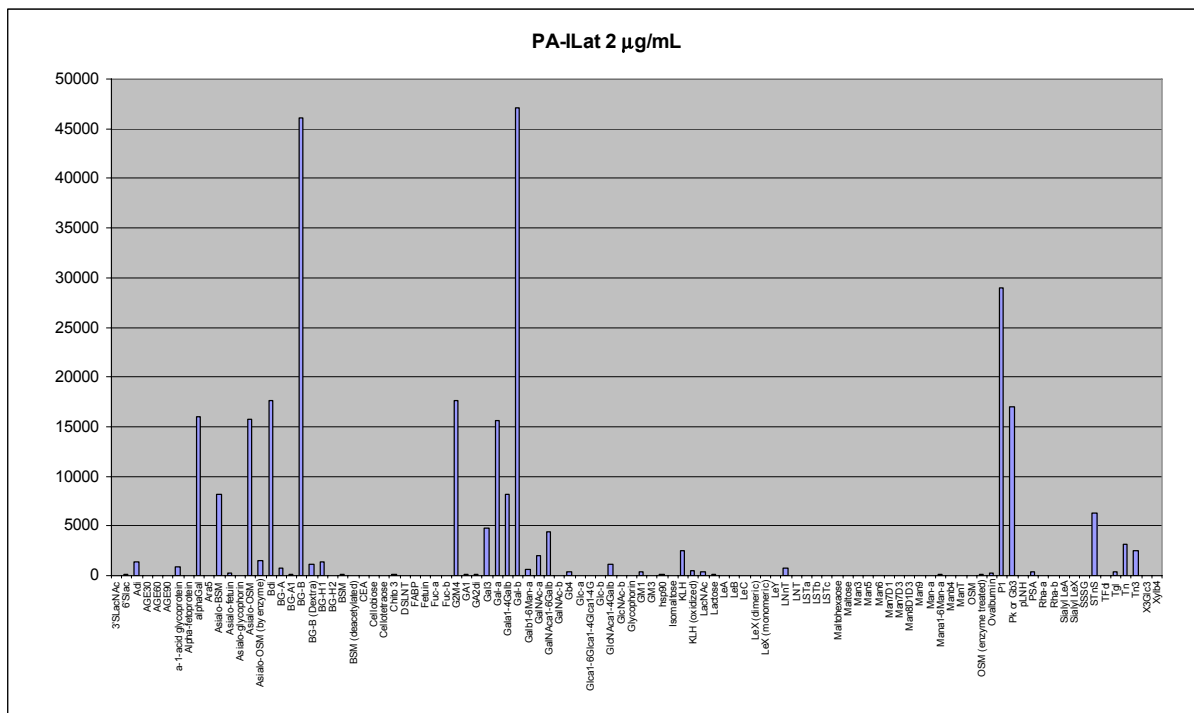
A.



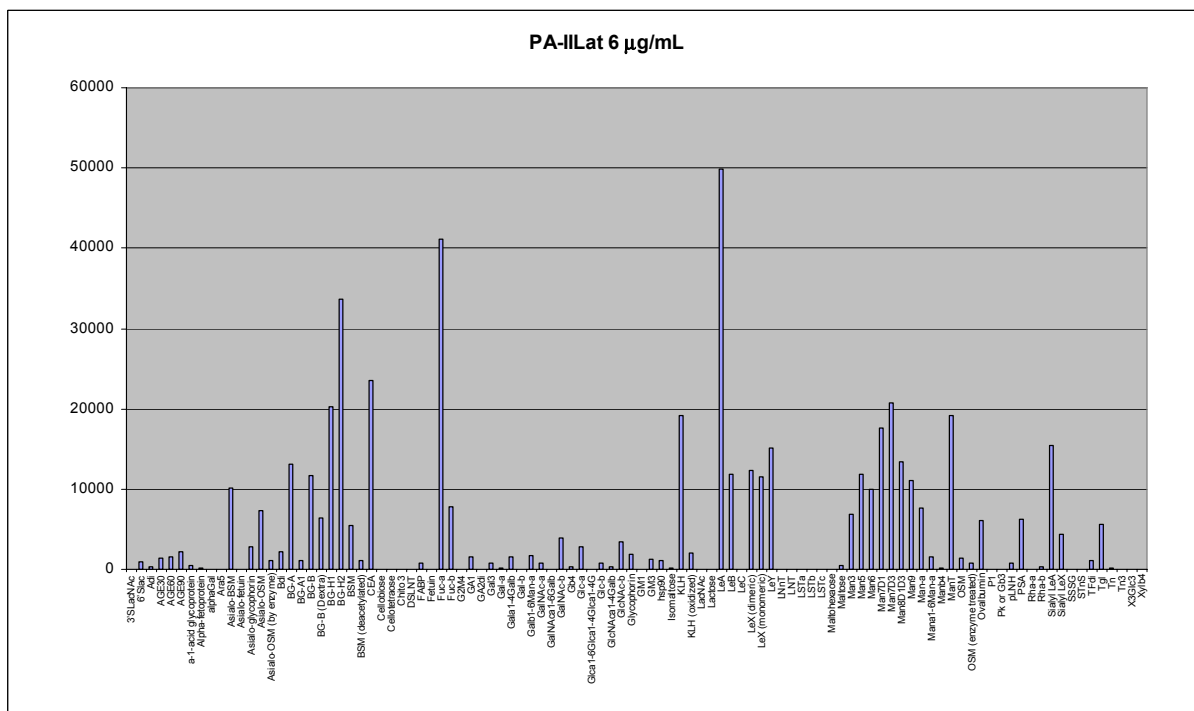
B.



C.

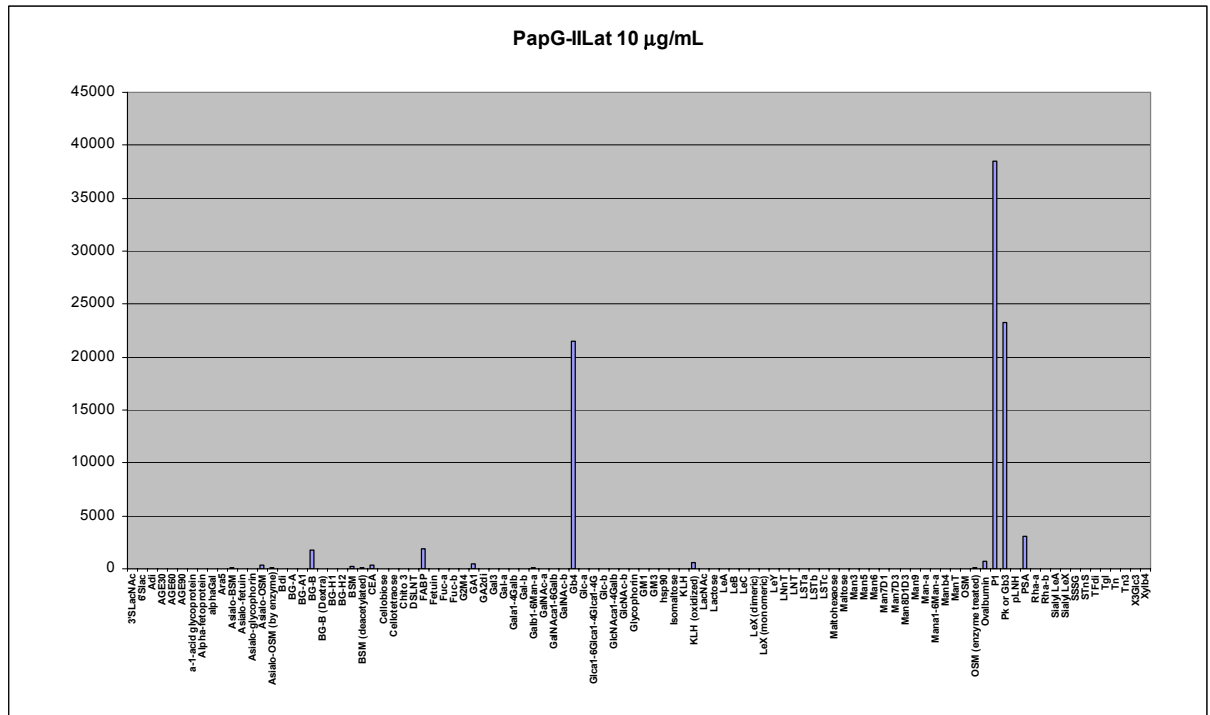


D.

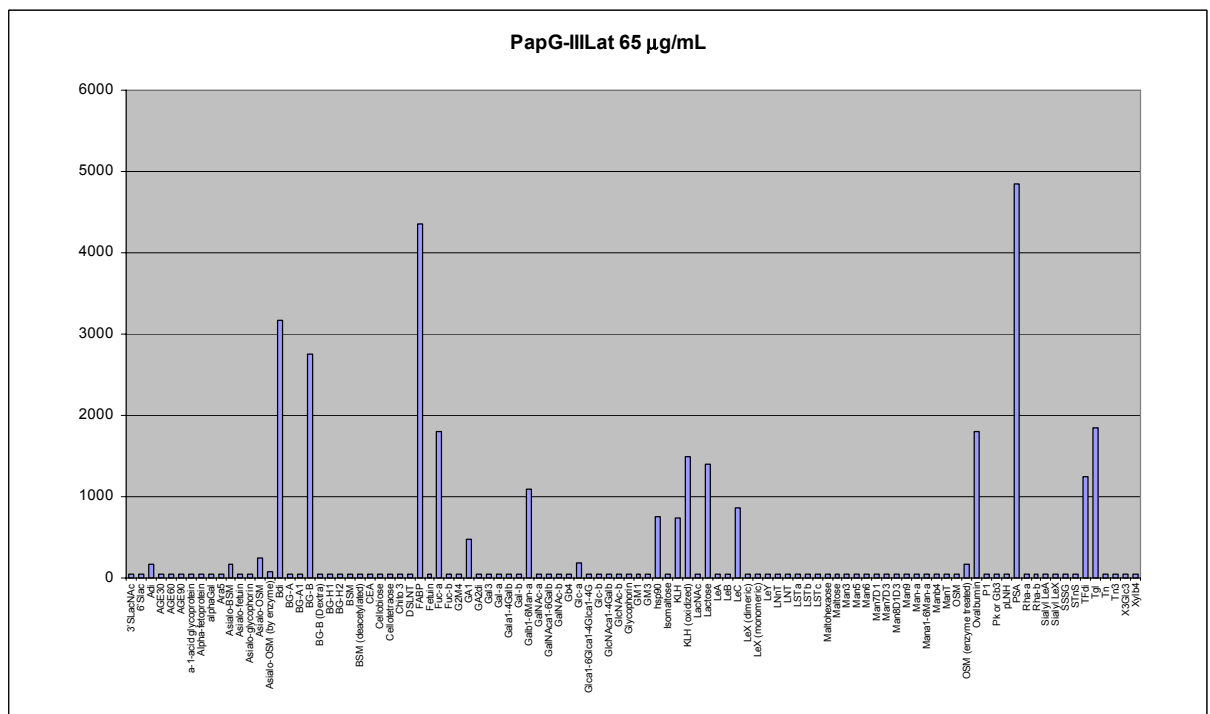




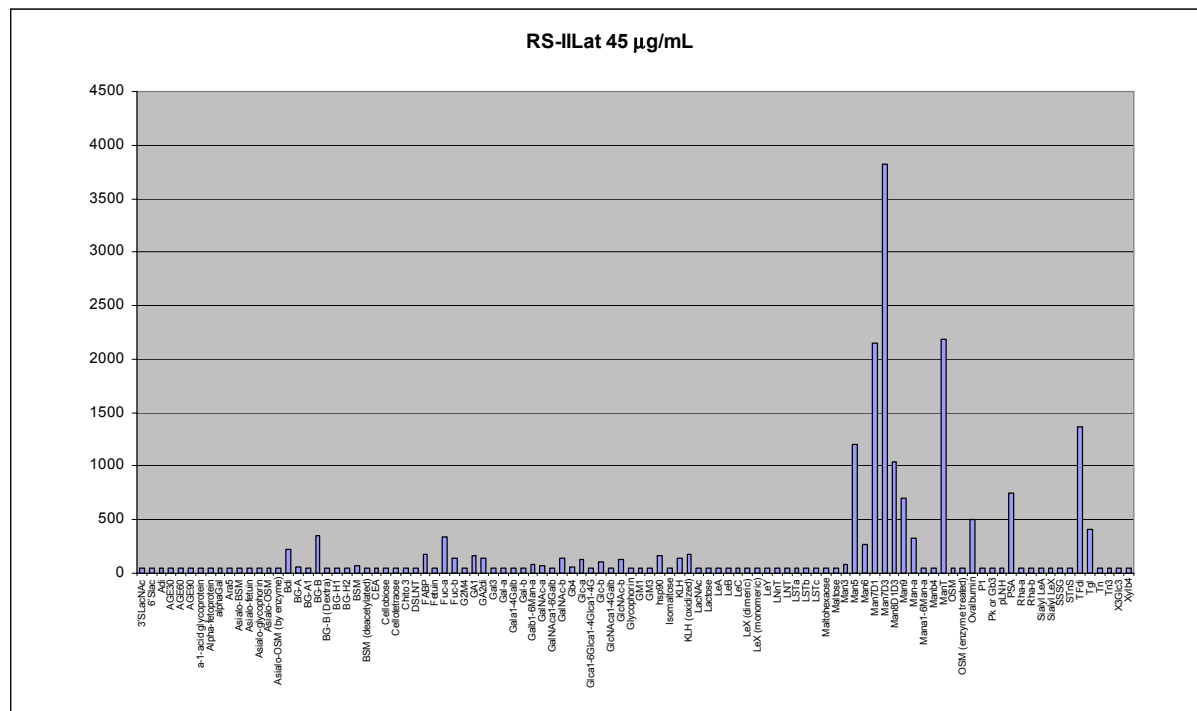
E.



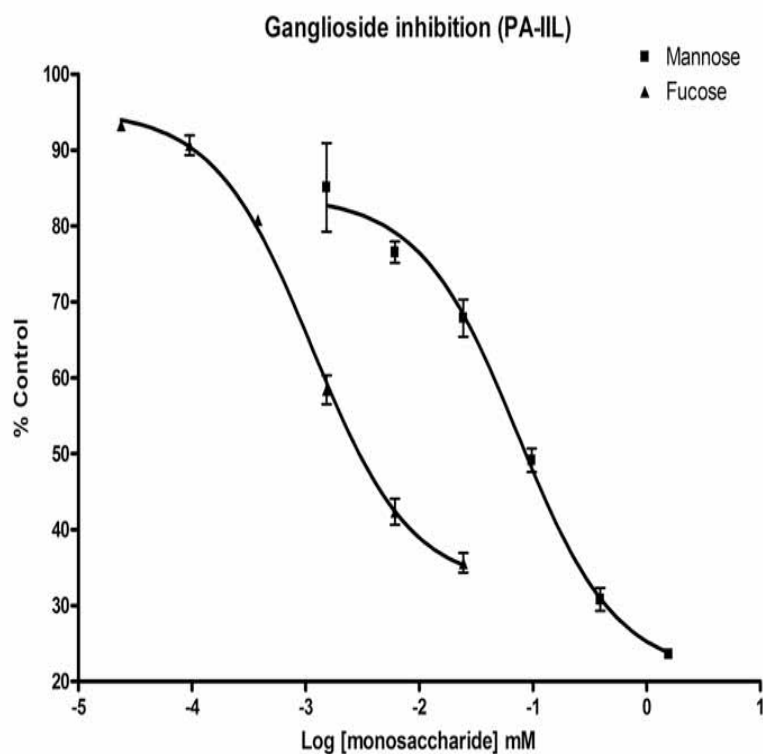
F.



G.

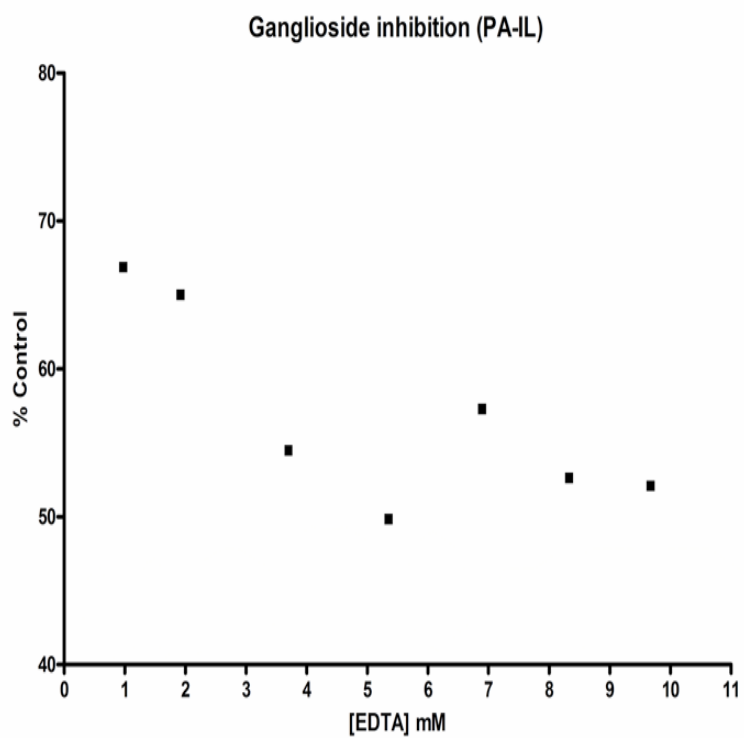


**Figure S2** Bar Graphs of the Carbohydrate Microarray Data Displayed in Figure 3. Lectin binding was detected by incubation with mouse anti-His5 antibody followed by Cy3-labeled goat anti-mouse antibody. For each graph, the y-axis is background corrected median fluorescence intensities and the x-axis contains individual array components. Lectins evaluated are as follows: A. GafD, B. GafD-m, C. PA-IL, D. PA-IIL, E. PapGII, F. PapGIII, G. RS-III.

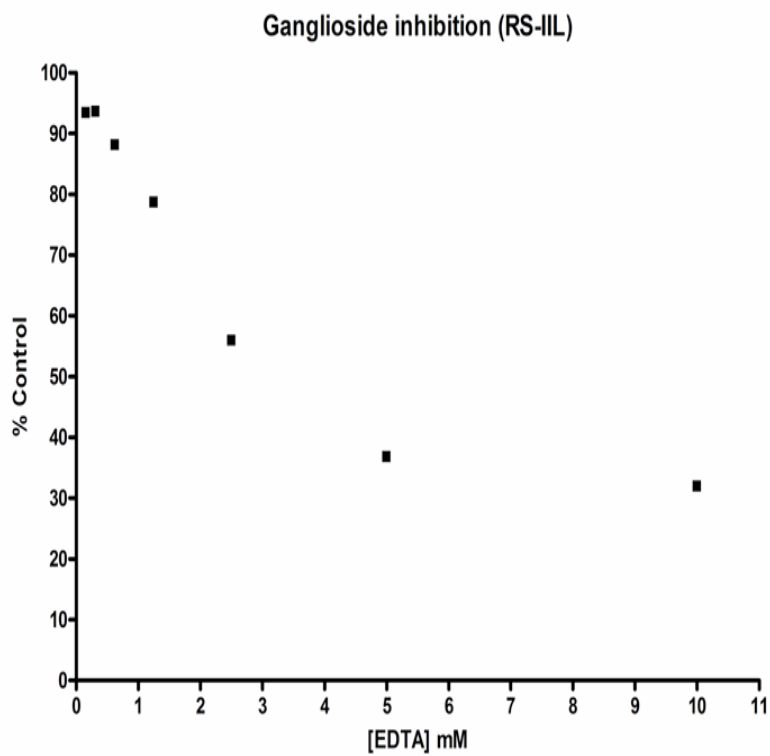


**Figure S3** *Inhibition of PA-IIL Binding to Gangliosides by ELISA Assay.* ELISA was run as described in *Experimental* with the following modification, varying concentrations of monosaccharide were incubated with PA-IIL (500 ng) prior to addition to the ELISA assay.  $IC_{50}$  values were calculated using non-linear regression analysis.

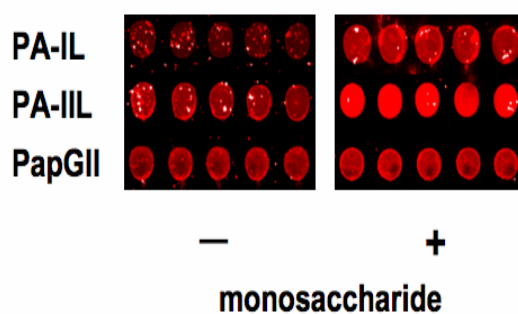
**A.**



**B.**

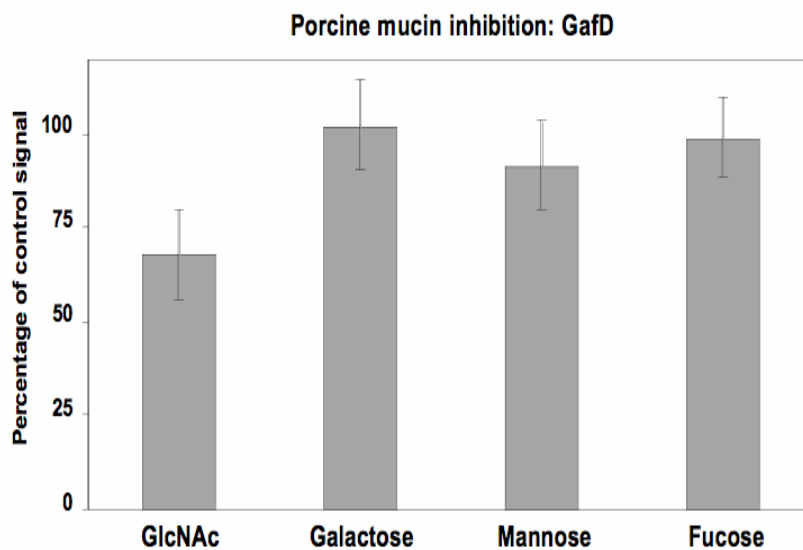


**Figure S4** *Inhibition of PA-IL and RS-III Binding to Gangliosides by ELISA Assay.* ELISA was run as described in *Experimental* with the following modification, varying concentrations of EDTA were incubated with lectins (500 ng) prior to addition to the ELISA assay.

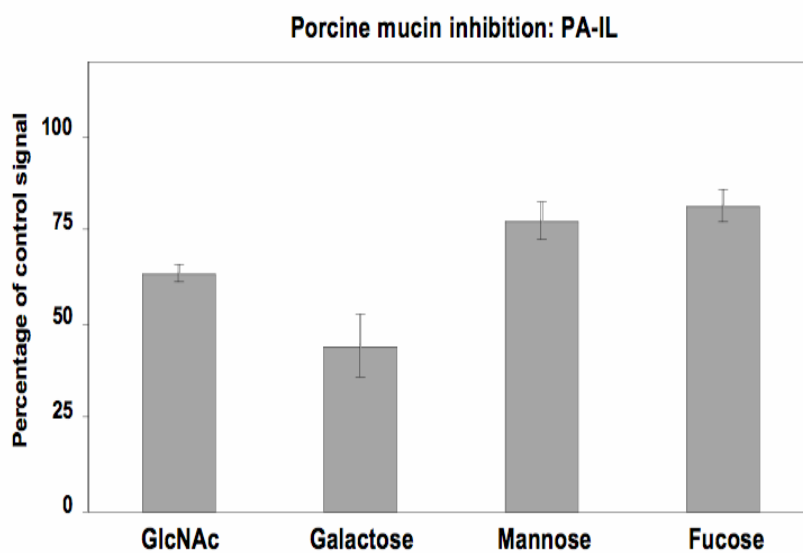


**Figure S5** *Spot Morphology and Lectin Activity are Improved by the Addition of Monosaccharides.* Lectins are printed as described in *Experimental* and probed with Cy5-Porcine Mucin (10  $\mu$ g).

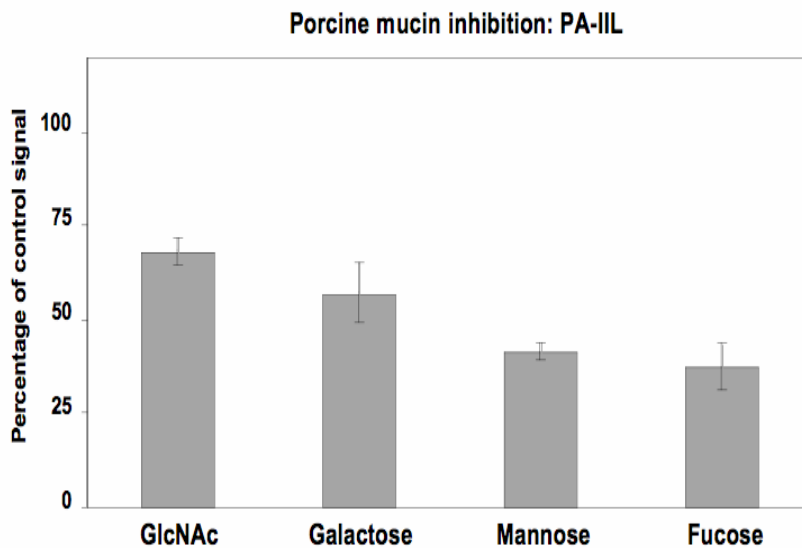
(a)



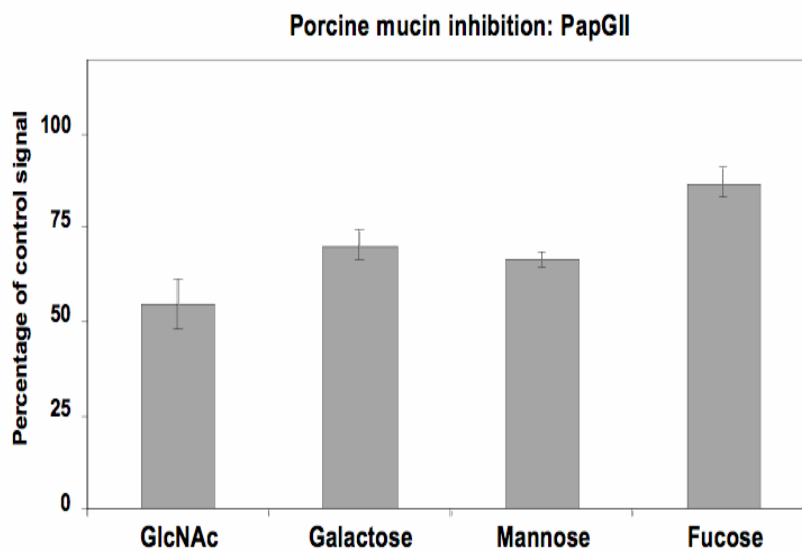
(b)



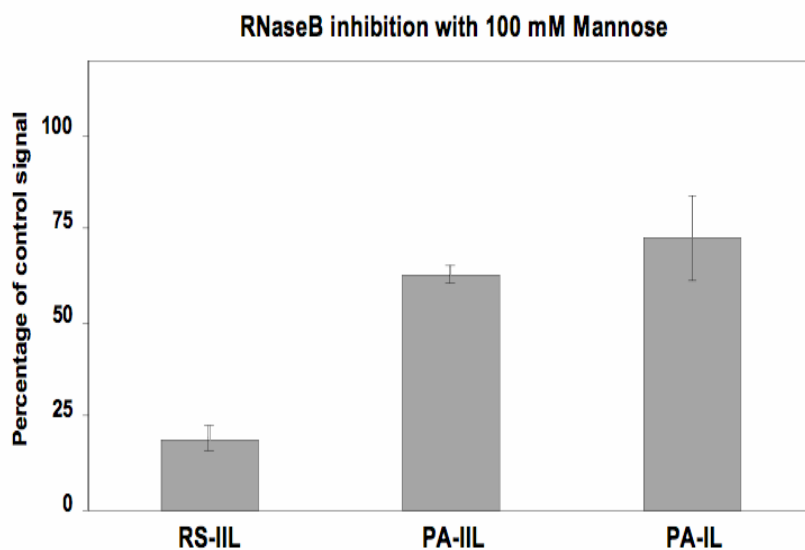
(c)



(d)



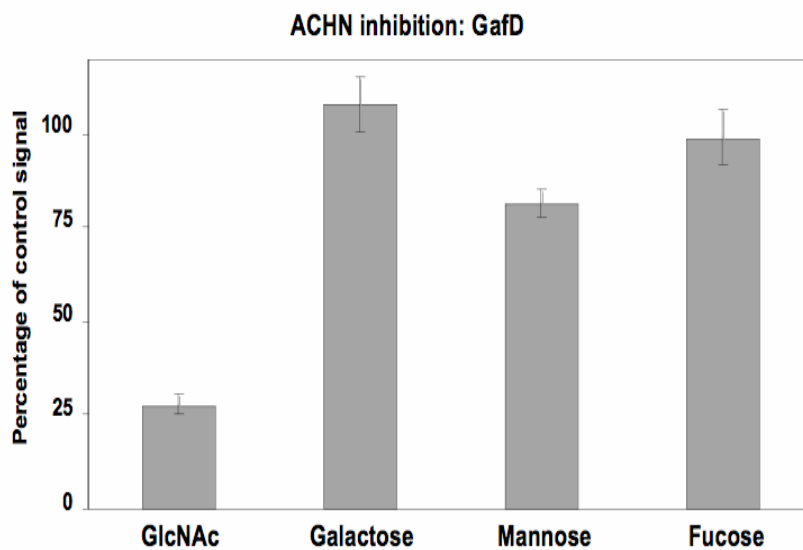
**Figure S6** *Inhibition of Porcine Mucin Signal by Monosaccharides.* Arrays were hybridized with 100 mM of indicated monosaccharide inhibitor for 30 min prior to addition of 10  $\mu$ g of Cy5-labeled Porcine Mucin. Inhibition data is shown for: (a) GafD, (b) PA-IL, (c) PA-IIL and (d) PapGII.



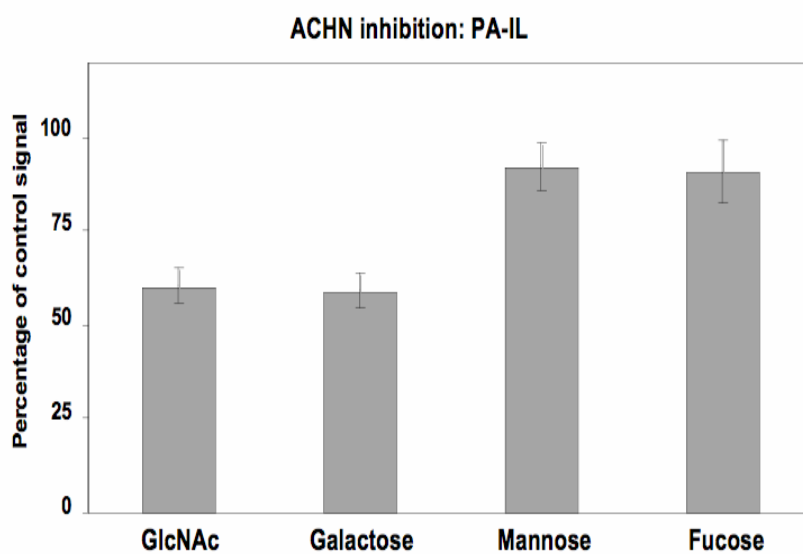
**Figure S7** *Inhibition of RNaseB Signal by Mannose.* Arrays were hybridized with 100 mM of mannose for 30 min prior to addition of 10  $\mu$ g of Cy5-labeled RNaseB.



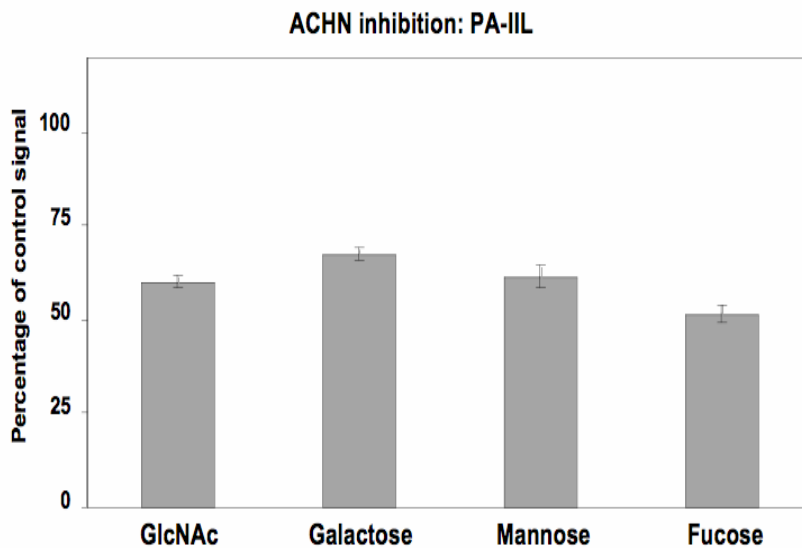
(a)



(b)



(c)



**Figure S8** *Inhibition of ACHN Cell Derived Signal by Monosaccharides.* Arrays were hybridized with 100 mM of indicated monosaccharide inhibitor for 30 min prior to addition of 10  $\mu$ g of Cy5-labeled cellular micellae derived from the ACHN cell line. Inhibition data is shown for: (a) GafD, (b) PA-IL and (c) PA-IIL.